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## Article

# Provenance Variation in Phenology and Frost Tolerance in Subalpine Fir (*Abies lasiocarpa*) Planted in Denmark and Iceland <sup>†</sup>

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<sup>†</sup> This paper is part of Brynjar Skulason's PhD thesis—Provenance variation in subalpine fir grown as an exotic tree species in Denmark and Iceland, from the Department of Geosciences and Natural Resource Management, Faculty of Science, University of Copenhagen, Denmark; delivered in February 2017.

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**Abstract:** In Iceland and Denmark, there is an interest in planting *Abies lasiocarpa* for use as Christmas trees. To search for usable genetic material for both countries, 26 provenances of subalpine fir, covering most of its natural range, were planted in eastern Iceland and Jutland, Denmark. Flushing, bud set and survival rates were assessed. Artificial freezing of twigs, from field trials in eastern Iceland and Denmark, was done to rank the provenances for frost tolerance in the spring and autumn. The northernmost provenances showed earliest bud set, highest autumn frost tolerance and a latitudinal cline was delineated. Differences between provenances in flushing and spring frost tolerance were less than that found for bud set and autumn frost tolerance. The southernmost provenances showed earliest flushing and the most spring frost damage on buds. Mortality of single provenances in the field tests could not be attributed to low freezing tolerances in the autumn or spring. The southernmost provenances of *Abies lasiocarpa* from New Mexico and Arizona showed the highest survival rate in the field trial in East Iceland, while the eastern provenances showed a low survival rate except for two provenances from Utah and Wyoming. The western provenances from Washington state showed the best survival in Denmark, followed by the southernmost provenances.

**Keywords:** provenance; *Abies lasiocarpa*; flushing; bud set; frost tolerance; survival; adaptation

## 1. Introduction

Subalpine fir (*Abies lasiocarpa* (Hook.) Nutt.) is grown as an exotic tree species in the Nordic countries. In Iceland and Denmark, there has been increasing interest in production of this fir as a Christmas tree. The first documented planting of subalpine fir in Denmark was in 1866 at Knuthenborg [1] but the species is still uncommon in plantations, parks and gardens [2]. Subalpine fir was introduced to Iceland from Denmark between 1903 and 1906 [3]. In Iceland and Denmark there is a long history of the use of exotic species in forestry [3,4]. Many of these species originated from North America or other parts of Europe [5]. Noble fir (*Abies procera* Rehder) and Nordmann fir (*Abies nordmanniana* (Steven) Spach) were initially introduced to Danish forests for timber production, but have become important in the production of Christmas trees and greenery [6–8]. In Denmark, subalpine fir could serve as an alternative Christmas tree species on exposed sites where production of Nordmann fir is unsuitable because of frost and as a niche product on the Danish market [9]. Also in Iceland the interest for subalpine fir is primarily related to Christmas tree production.

Subalpine fir is widely distributed in western North America, ranging in latitude from approximately 32° N to 64° N and growing in the coolest and wettest forests on the continent [10].

The distribution area is confined within a climatic envelope with a mean annual temperature of 3.4 to 4.8 °C, a mean temperature of the warmest month from 10.9 to 16.1 °C, a mean temperature of the coldest month of −23.2 to −5.9 °C, a mean annual precipitation of 365 to 1250 mm, and with degree days (DD) > 5 °C of 500 to 1200 DD [11]. Two varieties of subalpine fir are recognized. The common variety (*A. lasiocarpa* var. *lasiocarpa* (Hook.) Nutt.) is found north of Colorado, while corkbark fir (*A. lasiocarpa* var. *arizonica* (Merriam) Lemmon) grows in southern Colorado, Arizona and New Mexico. The large distribution area of subalpine fir indicates that the species can grow in a broad range of climatic conditions and might have large genetic and phenotypic variation [12]. The characteristics of a population may reflect adaptation to both present and past environments [13]. Alexander et al. [10] divided the distribution area of subalpine fir into four main regions, based on climatological data. The present distribution area may not yet have reached equilibrium with the climatic niche of the species after the last glacial maximum. Roberts and Hamann [14] reconstructed the refugial history of western North American trees and divided subalpine fir into 6 different refugia. Each provenance might have adapted partly to the present local climate, but the opportunity for adaptation may be constrained by evolution and dispersal from these refugia as well as genetic bottlenecks. Hansen et al. [15] used canonical variates analysis, based on 15 characteristics, to divide the 26 provenances into six groups. These groups show a strong relationship to the classification by Alexander et al. [10] and Roberts and Hamann [14].

In boreal forest trees, adaptation to seasonal climatic conditions is essential. Boreal tree species such as subalpine fir can tolerate extremely low temperatures (−80 °C) during winter dormancy [16], but temperatures slightly below freezing during the growing period can be lethal. The risk of cold injury is highest when trees are actively growing and frosts occur, for example a late spring frost around time of bud flush or in the early autumn during bud set before full cold acclimation [17–19].

The climate in Iceland is oceanic with rather mild winters and cool summers. The mean temperature in the coldest month of the year along the coast from southwest to southeast is above 0 °C. In low lying inland areas, especially in northern and eastern Iceland, frost can occur in all months [20]. The climate is highly variable and mean monthly temperatures can vary substantially between years. This variation is greater in the winter months than in the summer and increases with distance from the sea, especially in northern Iceland. The challenge facing Icelandic forestry is to choose genetic material that tolerates late spring frosts following mild winters as well as cool summers and early autumn frosts [21].

Compared to Iceland, Denmark generally has a higher annual mean temperature, a longer growing season and greater temperature differences between summer and winter (a more continental climate). Winter damage occurs occasionally in Christmas tree plantations of Noble fir and Nordmann fir [22,23]. In Denmark, because of the long growing seasons, autumn frosts are unlikely to damage subalpine fir, except for hallow inland areas. Mild weather in late winter followed by frost, is more likely to damage subalpine fir under Danish conditions.

In 1999, a Nordic project named “*Abies lasiocarpa* for Christmas tree production in the Nordic countries” was initiated. The broad objectives were to identify suitable provenances and develop cultivation methods to produce valuable Christmas trees of subalpine fir [9]. The results after three years in the field from provenance trials in Denmark, Norway and Iceland were published by Hansen et al. [15]. Part of this project was to identify variability in frost hardiness between provenances of subalpine fir and identify those provenances that are sensitive to spring and/or autumn frost [9].

The study presented here focused on the growth rhythms of the 26 provenances of subalpine fir in the Nordic project, originating from latitudes from 61° N in the Yukon to 34° N in New Mexico, thereby covering a large part of the species distribution. The following issues were examined and discussed:

- Provenance phenology for flushing and bud set
- Provenance ranking in frost sensitivity in late spring and early autumn based on tests in freezing chambers
- Relationships between date of flushing, spring frost tolerance, survival rates and the geographical origin of provenances
- Relationships between bud set, autumn frost tolerance, survival rates and the geographical origin of provenances.

## 2. Materials and Methods

### 2.1. Provenances and Sites

In the years 1991 to 1993, seeds of the 26 subalpine fir provenances (Table 1) currently evaluated were obtained from the USA and Canada [9]. These were cultivated at the Danish Tree Improvement Station at Humlebæk, Zealand, Denmark (Danish Nature Agency) as 3 + 2 bare-root plants (three years in seedbed after sowing and two years as transplants in a transplant line), and planted in provenance trials in three Nordic countries in spring 1999. The four trial sites in this study were established in Hallormsstadur, Iceland (65°14' N, 14°67' W, 35 meters above sea level (m a.s.l.)), Vilsbøl (55°10' N, 8°57' W, 48 m a.s.l.). Hønning (57°01' N, 8°35', 27 m a.s.l.) and Thorsø (56°09' N, 9°32' W, 100 m a.s.l.). The three latter sites are in Jutland, Denmark. Each trial contained 48 blocks with 28 single tree plots in each block—a total of initially 1344 seedlings at each site. Twenty-four provenances were represented once and two provenances represented twice in each block.

**Table 1.** The twenty-six provenances included in the joint Nordic provenance test. Provenances are listed in order of latitude, with the most northern provenances at the top. Provenance reference numbers, states from the USA and provinces in Canada, regional division according to Hansen et al. [15], latitude, longitude, elevation above sea level and provenance name are also shown. The provenances from New Mexico and Arizona are of the variety corkbark fir (*Abies lasiocarpa* var. *arizonica*).

Provenance No.	State/Province	Regional Division	Latitude/Longitude	Elevation m a.s.l.	Locality/Provenance
167	Yukon	North	61°50' / 133°00'	900	Canol Road
183	Alaska	North	59°50' / 135°30'	800	Seed zone A16, Skagway
166	Br. Col.	North	59°20' / 133°45'	800	Atlin
217	Br. Col.	West	55°48' / 129°10'	320	White River
168	Br. Col.	East	50°50' / 119°45'	1400	Adam's Plateau
218	Br. Col.	Central	50°43' / 115°27'	1525	Albert River
216	Br. Col.	Central	49°33' / 118°31'	1970	Blue Joint
219	Br. Col.	East	49°14' / 118°30'	1300	(Name unknown)
196 <sup>1</sup>	Wash.	West	48°37' / 120°36'	2000 <sup>2</sup>	Okanogan N.F., Mile Marker 172, Rt. 20
194 <sup>1</sup>	Wash.	West	48°29' / 119°58'	1300	Okanogan N.F., For. Rd. 42, Looploop Campg Rd.
195 <sup>1</sup>	Wash.	West	48°26' / 119°50'	1500	Okanogan N.F., For. Rd. 42, Rt.20
191 <sup>1</sup>	Montana	East	48°10' / 113°30'	1200	Flathead N.F., West of Marias Pass
170	Montana	East	48°00' / 115°30'	1200 <sup>2</sup>	Kootenai N.F.
171	Montana	East	48°00' / 114°15'	1100 <sup>2</sup>	Flathead N.F.
192 <sup>1</sup>	Wash.	West	47°55' / 117°07'	1400	Mount Spokane
193 <sup>1</sup>	Wash.	East	47°55' / 117°07'	1500	Mount Spokane
81	Montana	East	47°30' / 113°30'	1800 <sup>2</sup>	Flathead N.F., Hungry Horse
199 <sup>1</sup>	Wash.	West	46°47' / 121°40'	1200 <sup>2</sup>	Mt. Rainier N.F., Before Stevens Creek Cross.
182	Wash.	West	46°10' / 122°15'	1300	Seed zone 440, South-west of Mount St. Helens

Table 1. Cont.

Provenance No.	State/Province	Regional Division	Latitude/Longitude	Elevation m a.s.l.	Locality/Provenance
203 <sup>1</sup>	Wyoming	East	41°21' / 106°32'	2400	Medicine Bow N.F., East of F.S. Work Camp
201 <sup>1</sup>	Utah	East	40°15' / 112°00'	1500 <sup>2</sup>	Wahsatch N.F., Rt. Cottonwood Canyon
204 <sup>1</sup>	New Mexico	South	35°11' / 106°25'	2900	Cibola N.F., Sandria Crest
205 <sup>1</sup>	New Mexico	South	35°15' / 107°40'	2900	Cibola N.F., Mount Taylor
67	Arizona	South	34°00' / 109°38'	3000 <sup>2</sup>	Apache N.F., Hannagan Meadows
140	Arizona	South	34°00' / 109°38'	3000 <sup>2</sup>	Apache N.F., Hannagan Meadows + Big Lake
164	Arizona	South	34°00' / 109°38'	3000 <sup>2</sup>	Apache N.F., Hannagan Meadows + Big Lake

<sup>1</sup> Number of trees from which seed has been collected is five or fewer. <sup>2</sup> Approximate elevation.

## 2.2. Phenology

In the spring (April) of 2000 and 2010 flushing was scored once in the field trials in a) Hallormsstadur and Thorsø (earlier published by Hansen et al. [15]) and b) Vilsbøl and Hønning, respectively, analyzed as datasets from four sites (not averaged). Scoring was done following a scale developed by Langlet [24] (0: dormant buds; 1: buds slightly swollen; 2: buds more or less green; 3: burst of bud scales; 4: bud scales have been shed, needles still turned forward like a brush; 5: incipient shoot elongation, shoots and needles still soft; 6: shoots elongated, needles in final position).

In the autumn (August, September) of 2001, bud set was scored twice at each site, in Hallormsstadur and Thorsø [15], using the method described by Johnsen and Apeland [25] and Skrøppa [26] (0: no terminal bud; 1: tiny, white terminal bud; 2: terminal bud light brown, needles slightly darker green than the plants in class 1; 3: well developed bud scales, terminal buds larger than on plants in class 2). The data was analyzed as four datasets (two sites and two monitoring dates within sites, not averaged).

## 2.3. Survival

Survival was assessed in Hallormsstadur in 2011, 12 growing seasons after establishment and in Hønning in 2008, 10 growing seasons after establishment. The other two sites in Denmark showed low mortality and therefore are not as suitable as Hønning for analysis of survival.

## 2.4. Freezing Tests

For the first freezing tests in 2001, twigs were collected from the two-year-old provenance field trial (7 years from seed) in Hallormsstadur, Iceland. The timing of the collection and value of the freezing test temperatures were chosen to identify the maximum variation in freezing tolerances for all the material. On 7 May 2001, twigs were collected when the buds were swelling. Calculated degree days (>5 °C) from 1 January to date of collection in the spring were 33.5 for Hallormsstadur (data for Egilsstadir, from the Icelandic Meteorological Office in Reykjavík, Iceland). The twigs were about 7 cm long and taken from the current year's growth. Four twigs were taken from the second whorl from the top of each tree, one for each freezing treatment. Usually 15 trees from each provenance were sampled in this way. The twigs were kept in cold storage (approximately +6 °C) until the freezing tests were carried out on 9 May. Provenance Nos. 167, 216 and 164 were not included in the spring frost test due to lack of trees.

On 20 October 2012, a collection was done in Vilsbøl, Denmark. The twigs were the same size as those collected in Hallormsstadur (about 7 cm) and were taken from the green upper part of the crown. Usually 16 trees from each provenance were sampled. Five twigs from each tree were taken, corresponding to five freezing test temperatures, and kept in plastic bags in cold storage until freezing tests were carried out. Calculated photoperiod (day length, including civil twilight when the sun is

geometrically 6 degrees below the horizon) when collection of twigs was made in Vilsbøl, was 11 h and 25 min according to the website of Astronomical Applications—Department of the U.S. Naval Observatory [27]. As no frost occurred in the field during the autumn 2012 the observed freezing damage was from the test.

The twigs were placed into wet fresh sphagnum moss in the spring frost test 2001 and sprayed with water to prevent drying during the freezing process. Same method was used in the autumn frost test 2012 but then the twigs were placed into Sphagnum peat (M6, Kekkila Oy, Tuusula, Finland). Twigs collected from Hallormsstadur in 2001 were frozen to  $-4$ ,  $-8$ ,  $-12$  and  $-16$  °C in 4 computer-controlled freezers where the temperature was lowered 2 °C per hour until the minimum temperature was reached. Because of the small amount of parent material (small trees), no control was collected and included. The lowest temperature was maintained for 3 h and then raised 2 °C per hour. After thawing, the twigs were kept in high vapor, constant light and a temperature of about 20 °C. Scoring of the damage was done 2–3 weeks after freezing when the damage became visible. The same method was used for the twigs collected at Vilsbøl in the autumn of 2012 using freezing temperatures of  $-6$ ,  $-12$ ,  $-18$ ,  $-24$  °C and one treatment was maintained at 6 °C as a control.

Damage to cambium, needles and buds was scored visually by splitting the twigs lengthwise using a sharp knife. The damage to needles and/or cambium was assessed separately using the proportion of discolored and necrotic tissue rated on a scale developed by Johnsen [28] (0: no discoloration of needles or cambium; 1: up to ten per cent brown and/or discolored needles or cambium; 2–10: ten per cent intervals between classes of brown and/or discolored needles or cambium; 11: all needles or cambium completely brown). Freezing damage on buds was also evaluated by using a scale from 0 to 3 (0: no damage; 1: damage in the lateral buds; 2: damage in the terminal bud; 3: damage to both lateral and terminal buds).

## 2.5. Statistical Analysis

Statistical analysis was done using the statistical program R, version 3.0.1 [29]. The following model was used for an analysis to determine grade of flushing:

$$Y = \text{Site} + \text{Block (Site)} + \text{Provenance} + \text{Provenance} \times \text{Site} + \text{Error} \quad (1)$$

where Y is the observation of flushing on individual trees. Site and Block (Site) were assumed fixed effects, whereas Provenance, Provenance  $\times$  Site and Error were considered random. Another model was used to determine grade of bud set:

$$Y = \text{Site} + \text{Block (Site)} + \text{Time (Site)} + \text{Provenance} + \text{Provenance} \times \text{Site} + \text{Error} \quad (2)$$

where Y is the observation of bud set for individual trees. Site, Block (Site) and Time (Site) were assumed fixed effects, whereas Provenance, Provenance  $\times$  Site and Error were considered random. The “lmer” and “ranef” functions from the lme4 package [30] and the “anova” function from the lmerTest package [31] were used to predict flushing and bud set through best linear unbiased prediction (BLUP) values and to analyze the statistical model.

Homogeneity of variance was tested by Levene’s test and residual plots in R. Normal distribution was tested using the Shapiro-Wilks test and Q–Q plots. Flushing and bud set showed some deviation from normal distribution and some lack of homogeneity. Skewness and kurtosis for the distribution of flushing were  $-0.672$  and  $1.139$ , respectively. Corresponding values for bud set were  $0.153$  and  $-0.308$ , respectively. The conclusion was that the deviation from normal distribution did not affect the main results to any meaningful extent.

The data from the freezing tests consisted mostly of twigs with no damage within current tissue type or those totally damaged. Frost damage was therefore analyzed as binomial data (damaged or undamaged). The maximum likelihood logit model was used to estimate the probability of frost damage for the provenances within each freezing test and tissue type. Only results from each

freezing temperature showing closest to 50% damage were used for the estimation of the BLUP values. The following maximum likelihood logit model:

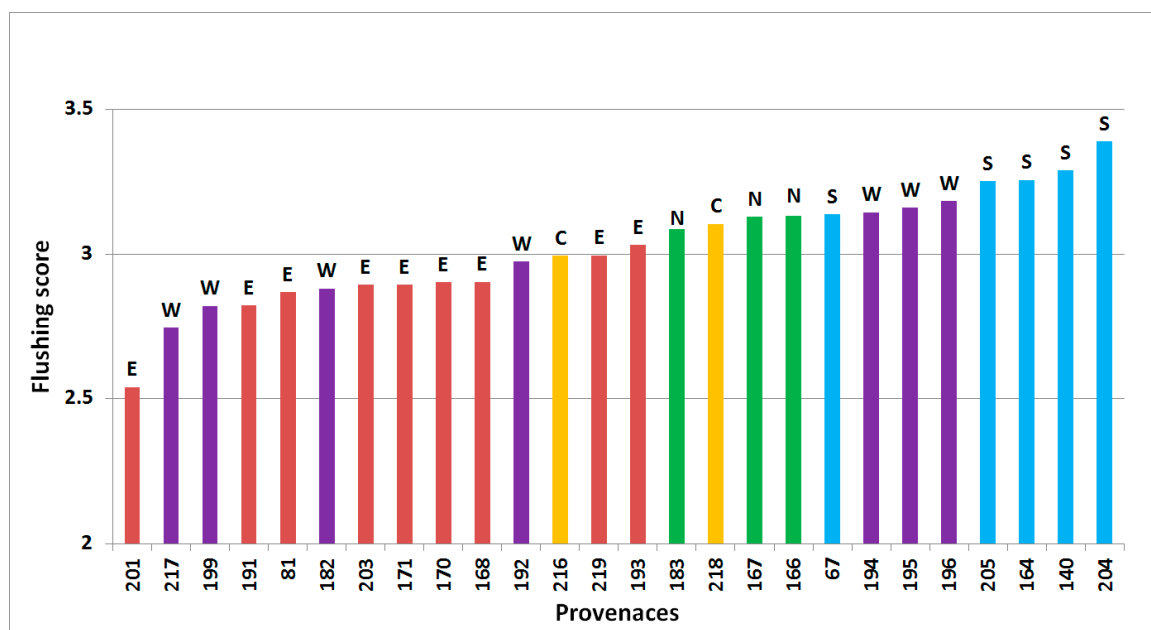
$$\text{logit}(P) = \log[P/(1 - P)] = \text{Block} + \text{Provenance} \quad (3)$$

was used for the data from 2012. In the model,  $P$  is the probability of damage on a twig depending on Block as a fixed factor and Provenance as a random factor. A similar model was used to estimate survival in the field trials in Hallormsstadur in the year 2011 and in Hønning 2008, where  $P$  is the probability of survival of the tree in the trial. Freezing data from the year 2001 (twigs collected from Hallormsstadur) had Provenance as a random factor with 15 replications within each provenance but no blocks. The “glmer” and “ranef” functions in the lme4 package [30], “glm” and “anova” from the stats package [32] and “Anova” from the car package [33] were used to calculate the significance of factors and predict values for the provenances. Q-Q plots were acceptable for all binomial variables. All values presented in the results section are best linear unbiased predicted (BLUP) values except Table 2 which are mean values. Predicted values from the logit model were back-transformed to per cent frost damaged scions before being presented as result values.

### 3. Results

#### 3.1. Phenology

Time of flushing varied significantly between site, provenance, block within site and interaction between site and provenance with  $p < 0.001$  (Figure 1). Flushing scores (scale developed by Langlet [24]) by provenance were significantly correlated across sites ( $p < 0.05$ , Pearson’s correlation coefficients 0.31 to 0.82) with one exception, between Hallormsstadur and Thorsø. No single provenance was a clear outlier.



**Figure 1.** Predicted flushing of 26 provenances of subalpine fir, based on assessment in Thorsø, Hallormsstadur (spring 2001), Hønning and Vilsbøl (spring 2010), arranged from latest flushing (left) to the earliest (right). Regional division according to Hansen et al. [15] is shown by colored bars and letters above bars (green/N = north, yellow/C = central, red/E = east, purple/W = west, blue/S = south).

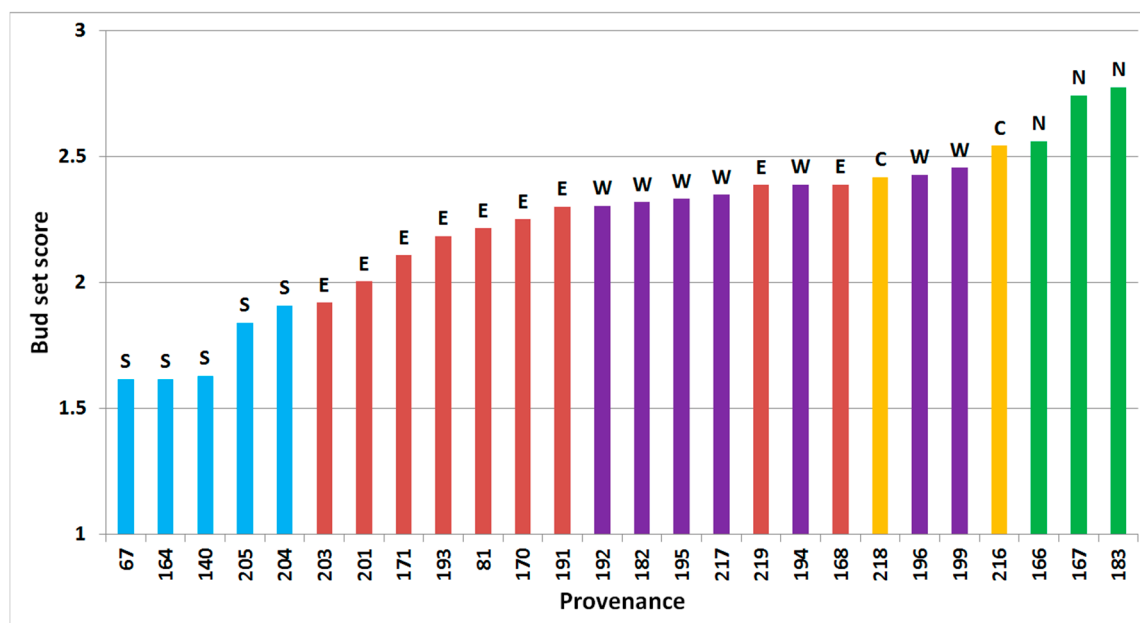


Bud set was significantly different between all tested factors of site, block within site, time of assessment within site, provenance and interaction between site and provenance (all  $p < 0.001$ , Figure 2). The Pearson's correlation coefficients between provenances means across sites and time of assessment were significant ( $p < 0.05$ ) and varied from 0.71 to 0.92.

### 3.2. Survival

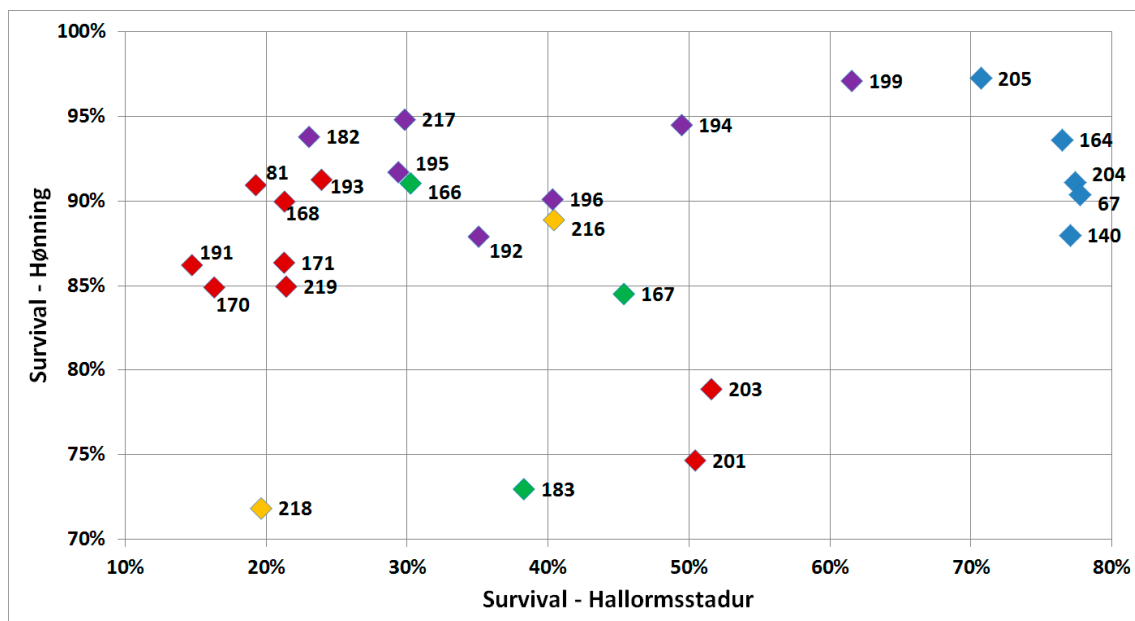
Overall average survival in Hallormsstadur for all provenances was down to 41% by 2011 (12 growing seasons after establishment). In the three Danish field trials the survival rate was much higher than in Hallormsstadur. Hønning showed the lowest survival rate with an average of 88 % (10 growing seasons after establishment).

Differences in survival between provenances were highly significant ( $p < 0.001$ ) within the sites, Hallormsstadur and Hønning. However, survival was not significantly correlated ( $r = 0.24$ ,  $p = 0.244$ ) between the sites (Figure 3). The southernmost provenances of corkbark fir, showed the highest survival rates in Hallormsstadur, while the eastern group showed the lowest survival rate with the exception of the two southeastern provenances Nos. 201 and 203 from Utah and Wyoming. These two provenances (201 and 203) showed on the other hand poorest survival in Hønning together with No. 183 from Alaska and No. 218 from British Columbia (Figure 3).



**Figure 2.** Predicted bud set of 26 provenances of subalpine fir, based on assessment in Thorsø and Hallormsstadur in the autumn of 2001, arranged from latest bud set (left) to the earliest (right). Regional division according to Hansen et al. [15] is shown by colored bars and letters above bars (green/N = north, yellow/C = central, red/E = east, purple/W = west, blue/S = south).





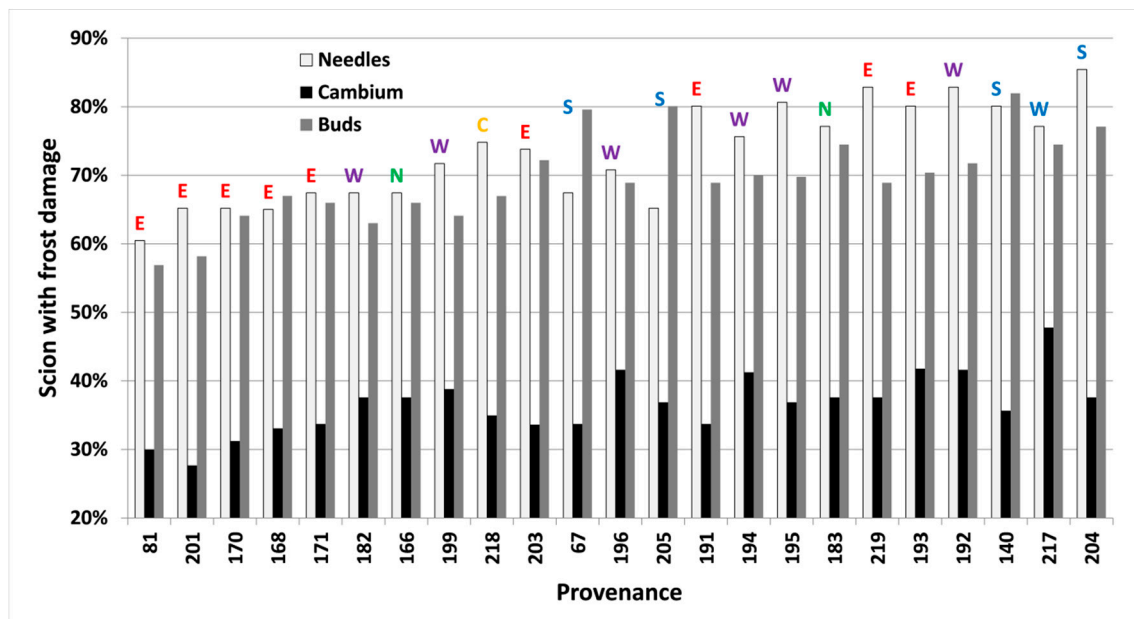
**Figure 3.** Predicted survival of various provenances in the field trial in Hønning (10 years after establishment) plotted against survival in Hallormsstadur (12 years after establishment). Regional division according to Hansen et al. [15] is shown by colored symbols (Green = North, Yellow = Central, Red = East, Purple = West, Blue = South).

### 3.3. Freezing Tests

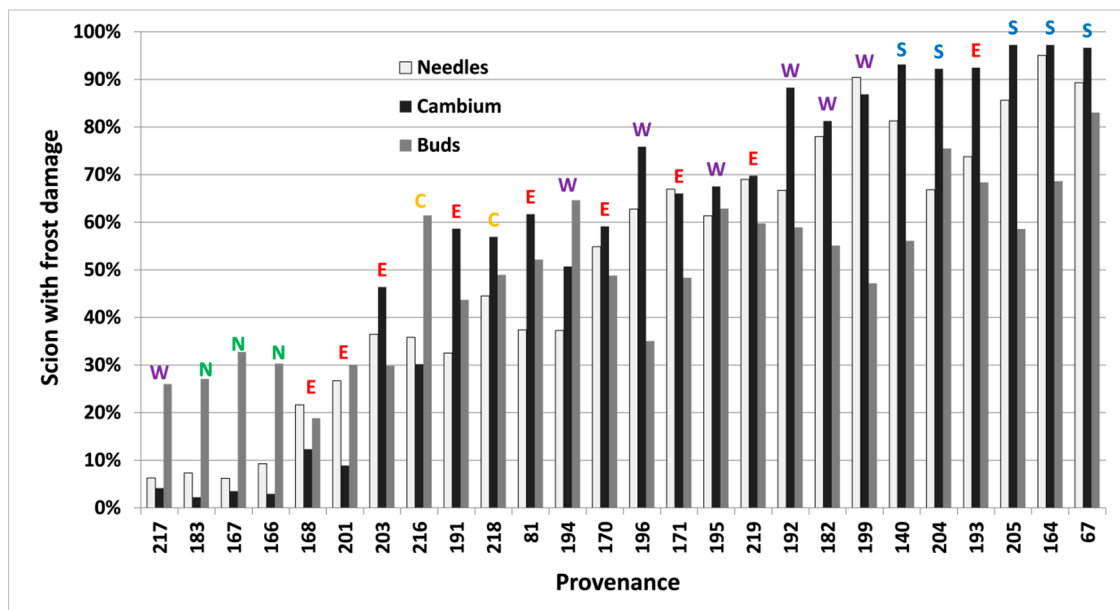
Frost damage both in spring and autumn differed significantly ( $p < 0.05$ ) between provenances, freezing temperatures and types of tissue in each freezing test. Buds were less frost resistant than needles and cambium in the autumn of 2012. Results closest to 50% within each test (Table 2, bold letters) were used to rank provenances further (Figures 4 and 5).

**Table 2.** Overall per cent of frost damage to twigs (mean values), depending on tissue type, date of freezing and test temperature. Results written in bold letters indicate the temperature for which data were used for further analyses to estimate freezing damage on various provenances.

Freezing Date	Temperature	Needles %	Cambium %	Buds %
Spring 2001	−4	9.5	2.4	14.6
	−8	14.0	3.0	21.5
	−12	22.2	3.5	28.9
	−16	<b>73.2</b>	<b>36.7</b>	<b>69.7</b>
Autumn 2012	4	1.0	1.0	1.2
	−6	0.5	0.5	0.5
	−12	2.9	3.9	9.5
	−18	21.5	29.8	<b>50.2</b>
	−24	<b>52.7</b>	<b>58.3</b>	99.8



**Figure 4.** Per cent of scions collected in 2001 in Hallormsstadur, with induced frost damage on needles, cambium and buds at  $-16^{\circ}\text{C}$  in spring, for 23 provenances of subalpine fir. Provenances are sorted by their lowest mean predicted proportion of damaged scions (left) across all tissue types to the highest (right). Regional division according to Hansen et al. [15] is shown by colored letters (green/N = north, yellow/C = central, red/E = east, purple/W = west, blue/S = south).



**Figure 5.** Per cent of scions collected in 2012 in Vilsbøl, with induced frost damage on needles, cambium and buds (at  $-24^{\circ}\text{C}$ ,  $-24^{\circ}\text{C}$  and  $-18^{\circ}\text{C}$ , respectively) in autumn, for 26 provenances of subalpine fir. Provenances are sorted by their lowest mean predicted proportion of damaged scions (left) across all tissue types to the highest (right). Regional division according to Hansen et al. [15] is shown by colored letters (green/N = north, yellow/C = central, red/E = east, purple/W = west, blue/S = south).

### 3.4. Correlations

Correlation of frost damage in the spring (Table 3) between various tissue types was positive but not always significant ( $p < 0.05$ ). Flushing and freezing damage on buds was positive and significantly correlated ( $p < 0.05$ ), indicating that early flushing increases the risk of frost damage on buds in the late spring. Provenances that flush early also showed significantly higher survival rates ( $p < 0.05$ ) in Hallormsstadur, indicating that spring frost damage was not responsible for the mortality in the field tests. Provenances from higher elevations flushed earlier and had more frost damage on their buds.

**Table 3.** Pearson's correlation coefficients between predicted frost damage for each provenance, tested in spring 2001 at  $-16\text{ }^{\circ}\text{C}$ , flushing, survival in Hallormsstadur 2011, survival in Hønning 2008 and geographical locations (latitude, longitude and elevation). Bold values are statistically significant ( $p < 0.05$ ).

	Needles $-16\text{ }^{\circ}\text{C}$	Cambium $-16\text{ }^{\circ}\text{C}$	Buds $-16\text{ }^{\circ}\text{C}$	Flushing
Cambium, $-16\text{ }^{\circ}\text{C}$	<b>0.52</b>			
Buds, $-16\text{ }^{\circ}\text{C}$	<b>0.49</b>	0.40		
Flushing	0.35	0.27	<b>0.67</b>	
Survival in Hallormsstadur	0.11	0.04	<b>0.63</b>	<b>0.50</b>
Survival in Hønning	$-0.06$	<b>0.45</b>	0.23	0.23
Latitude	0.01	0.32	$-0.40$	$-0.27$
Longitude	0.02	<b>0.47</b>	$-0.20$	$-0.13$
Elevation	0.01	$-0.26$	<b>0.54</b>	<b>0.56</b>

Frost damage on various tissue types in the autumn of 2012 (Table 4) were all significantly correlated ( $p < 0.05$ ). The damage on all tissue types was also significantly and negatively correlated with bud set as expected (earlier bud set results in less autumn frost damage). Provenances originating from high latitudes showed significantly less frost damage in autumn and earlier bud set. Surprisingly, survival at both sites had a positive correlation with tissue damage and negative correlation with bud set, indicating that autumn frost hardiness is not an important characteristic for survival in Hallormsstadur and Hønning. Bud set and frost damage in all tissue types in autumn showed a high correlation with latitude, longitude and elevation, indicating that northern provenances have early bud set and high freezing tolerance in autumn.

**Table 4.** Pearson's correlation coefficients between predicted frost damage for each provenance tested in the autumn of 2012, predictions for bud set, predictions for survival in Hallormsstadur (Hall.) 2011, Hønning 2008 and geographical locations (latitude, longitude and elevation). Bold values are statistically significant ( $p < 0.05$ ).

	Needles $-24\text{ }^{\circ}\text{C}$	Cambium $-24\text{ }^{\circ}\text{C}$	Buds $-18\text{ }^{\circ}\text{C}$	Bud Set	Survival Hall.	Survival Hønning
Cambium, $-24\text{ }^{\circ}\text{C}$	<b>0.95</b>					
Buds, $-18\text{ }^{\circ}\text{C}$	<b>0.73</b>	<b>0.78</b>				
Bud set	$-0.63$	$-0.62$	$-0.50$			
Survival in Hall.	<b>0.43</b>	0.37	0.38	$-0.62$		
Survival in Hønning	<b>0.42</b>	<b>0.42</b>	0.38	$-0.19$	0.24	
Latitude	$-0.74$	$-0.72$	$-0.59$	<b>0.93</b>	$-0.68$	$-0.22$
Longitude	$-0.61$	$-0.66$	$-0.52$	<b>0.84</b>	$-0.40$	$-0.05$
Elevation	<b>0.63</b>	<b>0.64</b>	<b>0.57</b>	$-0.82$	<b>0.75</b>	<b>0.17</b>

## 4. Discussion

### 4.1. Methods and Assessment

The timing and methods employed in freezing tests are critical to the possibility of ranking the provenances based on frost resistance in spring and autumn. In full dormancy, subalpine fir tolerates much lower temperatures than can be expected in Iceland and Denmark [16], but in the active growing

period it only tolerates a few degrees below freezing [34]. When the goal of the testing is to rank the provenances, it is desirable to use data for which the mean is closest to 50% of the twigs damaged, in order to obtain the maximum resolution of measurements using the scale. The analyzed freezing tests in the spring of 2001 and autumn of 2012 showed results with an average close to 50% damage, especially in the autumn (Table 2). Because a control was not included in the freezing test in 2001, some minor older damage on some scions before freezing cannot be precluded.

Flushing and bud set were assessed directly on the trees in the field, but for practical reasons twigs from trees in the field were used in artificial freezing to study frost tolerance in spring and autumn. Vitasse and Basler [35] compared budburst in adult trees, seedlings and twigs of three tree species (*Carpinus betulus* L., *Acer pseudoplatanus* L., *Fagus sylvatica* L.). They found that the twigs were better surrogates than juvenile trees to assess the phenology of adult trees, but all three methods gave similar results. Cold hardiness is under strong genetic control. It is common to use relative methods to screen for cold hardiness and it is considered to have a high correlation with field performance [36]. For instance, twigs were used by Basler and Körner [37] in a study of budburst in 14 temperate forest tree species in growth chambers. Eysteinnsson et al. [38] used twigs to rank provenances of four species of larch (*Larix sukaczewii* Dyl., *L. sibirica* Ledeb., *L. gmelinii* Rupr. and *L. cajanderi* Mayr.) for cold hardiness in spring and autumn. Beuker et al. [39] used twigs to map seasonal variation in frost hardiness in various provenances of Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* (L.) Karst.) and Nielsen [40] also used twigs when testing for cold hardiness for various provenances of Nordmann fir (*Abies nordmanniana*).

#### 4.2. Bud Set and Autumn Frost Hardiness

A significant relationship was found between bud set and frost damage to various tissue types in autumn (Table 4). All data from the autumn showed clear differences between provenances and an obvious geographical cline. The provenances originating from higher latitudes and lower temperatures in winter showed earlier bud set and increasing autumn frost hardiness. This is a general tendency in North American tree species where the northernmost areas are coldest with short growing seasons and the earliest bud set [41]. This relationship between latitude, low winter temperatures and time of bud set is common in the boreal zone among conifers with large distribution areas, for example Douglas fir (*Pseudotsuga menziesii* Mirb.) [42], Lodgepole pine (*Pinus contorta* Dougl. var. *latifolia* Engelm.) [43] and Siberian larch (*Larix sibirica*) [38]. Generally, needles and cambium were harder than buds in both tests (Table 2). Burr et al. [44] found a similar pattern for ponderosa pine (*Pinus ponderosa* P. and C. Lawson), Douglas fir and Engelmann spruce (*Picea engelmannii* Parry ex Engelm.) where buds were consistently the least cold hardy of the three tissue types. Sakai and Weiser [41] found the winter hardiness of buds of subalpine fir and white fir (*Abies concolor* (Gordon) Lindl. ex Hildebr.) only reached  $-40\text{ }^{\circ}\text{C}$ , but corresponding tolerance for needles and cambium was below  $-80\text{ }^{\circ}\text{C}$ . Ranking of tissue types by cold hardiness may vary during the annual growth cycle and it is important to assess these separately [41,45]. Early bud set might be an essential characteristic in interior areas of Iceland where the growing season is short and first frosts occur early. Early autumn frosts should be less of a problem in Denmark than in Iceland because the growing season is typically longer with exception for unusual frost exposed areas.

#### 4.3. Flushing and Spring Frost Hardiness

Compared to other fir species, subalpine fir is known for early budbreak and is easily damaged by late frosts in spring, especially in an environment with fluctuating winter temperatures [46,47]. In the oceanic climates of Iceland and Denmark, an unseasonable flushing with high risks of spring frost damage would be expected because of high winter temperatures when compared to the climate to which subalpine fir is adapted [11]. The correlation between spring frost tolerance and flushing was positive, but only significant for damage on the buds. Later freezing might have produced greater differences in frost damage between provenances. The result for flushing and spring frost damage

showed less difference between provenances compared to the results for bud set and autumn frost damage. Barney et al. [48] assessed flushing dates on both common subalpine fir (var. *lasiocarpa*) and corkbark fir (var. *arizonica*) for three years. They found that the former broke bud less than 1.5 days earlier than corkbark fir, indicating only a small difference between the two. In our study, the corkbark fir provenances flushed first, then three provenances originating from Okanogan N.F. in the Cascade Range and thereafter the northern group. Provenance No. 201 flushed latest and was the most frost tolerant in the spring for all tissue types.

Generally, cold hardiness and bud set phenology in autumn show stronger population differentiation and clines along provenance thermal gradients with less phenotypic plasticity than flushing and spring frost tolerance [13,49–51]. Flushing is dependent upon the interaction of the accumulation of cold sums and temperature sums above a specific threshold value. Critical temperature sums and thresholds for flushing vary among species and populations within the species [47,51,52]. Photoperiod length may also influence the timing of flushing [37]. Worrall [47] studied flushing in subalpine fir at various elevations and found that both the required heat sum and threshold value for flushing is low for this species. He also found that the threshold values are lower in populations from high elevations (2.4 °C) as compared to populations from low elevations (2.8 °C) in the same region. Green [53] also found subalpine fir from high elevations flushed earlier than populations from lower elevations, when they were exposed to the same temperatures. Low threshold values with changing elevations is important in oceanic climates similar to those in Iceland and Denmark, where temperatures in late winter commonly fluctuate between 0 and 5 °C. Green [53,54] compared flushing and growth in interior spruces (white spruce and Engelmann spruce), lodgepole pine and subalpine fir from various elevations, cultivated in greenhouses and in the field. The timing of growth initiation for subalpine fir was intermediate between lodgepole pine (earliest) and interior spruce in the field, but similar to interior spruce when cultivated in a greenhouse. Higher temperature sums were required for flushing in the greenhouse than in the field for subalpine fir, indicating photoperiodic control of flushing for the species. Basler and Körner [37] also found an effect of long day treatment of silver fir (*Abies alba* Mill.) on early flushing. Requirements for chilling may delay flushing in many boreal tree species when winter temperatures become warmer or when a tree species or provenance is moved into a new environment with warmer winter temperatures [51,52,55]. Harrington and Gould [56] studied chilling requirements of Pacific Northwest tree species and found Pacific silver fir (*Abies amabilis* Douglas ex J.Forbes), grand fir (*Abies grandis* (Douglas ex D.Don) Lindl.) and noble fir all had obligate chilling requirements. The same may apply to subalpine fir.

The variation in spring frost tolerance in subalpine fir based on our study and the discussion above reflects complex patterns of local adaption in spring. New freezing tests done in the spring after 50–70 degree days (>5 °C) with shorter intervals between freezing temperatures might identify further differences between and within provenances that makes selection and breeding for this important characteristic more effective.

#### 4.4. Frost Hardiness and Survival

Surprisingly, provenances demonstrating frost hardiness in the late spring or early autumn did not show higher survival rates in either Hallormsstadur or Hønning. A crown shelter of Siberian larch at Hallormsstadur may have prevented frost damage in the trial plants. The field trials in both countries might have avoided from early autumn frosts during the test period or the interplay between high winter temperatures and damaging spring frosts. The relationship between survival and spring frost hardiness may be different in even more oceanic climates. There may also be a positive correlation between survival and autumn frost hardiness when the growing period is shorter, with earlier autumn frost or at more frost exposed sites than at our test sites. Varying chilling requirements of the provenances were not tested in this research, but could prove an important factor for avoiding premature flushing in winter in areas with oceanic climates.

Survival in refugia in the past and migration pathways after the last glacial retreat may be important factors in forming populations with differing characteristics. The results in our trials for survival, flushing and bud set showed similar geographical groupings as those found by Hansen et al. [15]. There were also similarities with the division into refugia done by Roberts and Hamann [14]. Taken together, this indicates that the selection of favorable genetic material for use in Iceland and Denmark should concentrate on geographical groups of provenances from certain refugia rather than frost hardiness in spring and autumn. The western provenances from Washington state are recommended for Denmark and the southern provenances from the mountains in New Mexico and Arizona are recommended for Iceland.

## 5. Conclusions

There was a clear geographical grouping of provenances by flushing, bud set, survival and autumn frost tolerance. The northernmost provenances showed early bud set and greatest autumn frost tolerance. There was a consistent significant correlation between autumn frost tolerance in all tissue types and bud set. Grouping of provenances and correlations between flushing and spring frost tolerance were less defined than results from the autumn. The survival rates in Hallormsstadur and Hønning were not significantly correlated and provenances showing sensitivity for spring or autumn frosts did not show any signs of higher mortality at either site in this study than did more frost hardy provenances.

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